

Comments on Intracellular Studies of Presynaptic Inhibition*

ALFRED J. SZUMSKI

Department of Physiology, Medical College of Virginia, Richmond

The use of intracellular electrodes, e.g., (micropipettes), in electrophysiological studies of the central nervous system, has enhanced our understanding of the basic function of the nervous system. The purpose of this paper is to review a study in which this microtechnique was successfully employed in the spinal cord.

INTRODUCTION

When the tip of a microelectrode impales a motoneurone innervating a dissected muscle under study, certain responses can be recorded which indicate that the neurone has been "engaged," and agonist and antagonist influence on the neurone may be investigated. Criteria for evaluation of the response of the cell are: (1) shifts of the average level of the membrane potential in a hyperpolarizing (inhibitory) or depolarizing (facilitory) direction,

(2) monosynaptic postsynaptic potential changes during neurone excitability tests, (3) synaptic activation "noise" (low voltage miniature potentials) which may spike in a hyperpolarizing or depolarizing direction and (4) alteration of the firing rate of a motoneurone stimulated by transmembrane currents passed through the tip of the impaling microelectrode. The first two criteria have been described by Eccles and co-workers (1964) in their intracellular studies; the latter two were first described for this type of investigation by Granit, Kellerth, and Williams (1964 a and b).

An evaluation of the above criteria by Granit and co-workers (Granit, Kellerth, and Williams 1964a and b) has shown that the recorded response of the neurone to stretch may be variable in relation to membrane potential and monosynaptic test response, but characteristic synaptic activation noise apparently depended on how near the microelectrode tip was to the source, i.e., the activated portion of the cell membrane. However, when the motoneurone was fired by transmembrane current and its muscle was stretched, the cell fired at an increased frequency. When an antagonist muscle was stretched, criteria (1) and (2) could be variable as is seen for the agonist, but criterion (4), in this case, showed a dramatic inhibition of the firing of the neurone. Since the cell membrane is directly affected by the "injected" transmembrane current, the response (facilitation or inhibition)

is postsynaptic. Therefore, Dr. Kellerth and I decided to use the above criteria, especially criteria (3) and (4), to determine if these responses are relevant in identifying a type of synaptic inhibition described by Eccles (1964) as presynaptic inhibition. For this type of inhibition, these authors proposed that the synapse is located on an excitatory synaptic terminal (endbulb), and that these axo-axonal synapses act by depolarizing the synaptic terminals, thereby diminishing their release of excitatory transmitter substance and, in turn, diminishing the size of the postsynaptic impulses (the excitatory postsynaptic potential), and resulting in an inhibitory effect. Since this proposed inhibitory action occurs primarily on the presynaptic terminals, it has been called presynaptic inhibition.

The argument for the occurrence of the presynaptic-type of inhibition is based mainly on studies where the afferent inflow to the neurone has been induced by electrical stimulation of the peripheral nerves and the effects of these synchronous volleys were recorded intracellularly. The criteria for inhibition were: (1) decreased monosynaptic excitability as measured by a diminution of the excitatory postsynaptic potential (EPSP) or (2) hyperpolarization of the postsynaptic membrane, or both. A reduction in the size of the EPSP without or with only a negligible shift of the membrane potential in a hyperpolarizing direction sug-

* The work discussed here was conducted in the laboratory of Professor Ragner Granit at the Nobel Institute for Neurophysiology, Karolinska Institute, Stockholm, Sweden, with the collaboration of Swedish Medical Candidate Jan-Olof Kellerth. The research was done during my tenure of a Postdoctoral Fellowship from the Vocational Rehabilitation Administration, H.E.W. (1964-1965). This experience was possible through the efforts of Drs. Ernst Fischer and Robert Ramsey. I am grateful for their support and encouragement during the academic years and the postdoctoral program.

gested a presynaptic mechanism.

Further elucidation of the possible mechanism of presynaptic inhibition was provided by results from pharmacological investigations which suggested that presynaptic and postsynaptic inhibitions differ in their response to certain convulsive drugs: strychnine was found to eliminate postsynaptic inhibition, while the presynaptic component was left intact or even slightly enhanced, and picrotoxin had no effect on postsynaptic inhibition although it reduced presynaptic inhibition (Eccles, Schmidt, and Willis, 1963).

Granit, Kellerth, and Williams (1964a and b) showed that when a natural (asynchronous) stimulus was used to initiate the afferent volley to the neurone, criterion (4), i.e., transmembrane current stimulation, was the only reliable method of revealing excitability changes. We therefore decided to further examine the validity of using strychnine and picrotoxin to differentiate between presynaptic and postsynaptic spinal cord inhibitions.

METHODS

The effects of afferent impulses from peripheral muscle receptors on spinal cord motoneurons in response to natural stimulation (muscle stretch) were studied in anesthetized cats (pentobarbitone, 35 mg/kg). Certain flexor and extensor hindlimb muscles were dissected so that their nerves were kept intact, and the muscle insertions could be attached to a strain gauge which recorded stretch or contraction.

Stimulating electrodes were placed on the nerves supplying these muscles and their cut ventral roots. Lumbosacral motoneurons were impaled with glass microelectrodes filled with 2M-potassium citrate, and a bridge circuit was so arranged that simultaneous records of transmembrane voltage changes could be recorded, and polarizing currents could be "injected" into the cell through the microelectrode.

These methods are more completely described by Granit, Kellerth, and Williams (1964a and b). Postsynaptic inhibitions were identified using the criteria described above, and the effects of intravenously administered strychnine, picrotoxin or both were evaluated. Overt convulsive movements were controlled with Flaxedil (Kellerth and Szumski, 1966a and b).

RESULTS AND COMMENTS

Motoneurons were selected which responded with maintained discharge frequency during a long-lasting (14 to 40 sec) injection of depolarizing current. The unequivocal effect of muscle stretch on repetitive firing was first tested since it was the most sensitive criterion for inhibition. However, synaptic activation noise, shifts of the average level of the membrane potential, and monosynaptic EPSP size also were recorded and were generally found to respond in the same direction; in a hyperpolarizing direction during an inhibitory response and in a depolarizing direction during facilitation. In some instances, the synaptic activation noise wavelets were not recorded in a predominantly hyperpolarizing or depolarizing direction, and membrane potential shifts were minimal. This result indicated that the tip of the microelectrode was not always near enough to the source of the event.

With microelectrodes definitely recording postsynaptic events within a neurone during muscle stretch, a convulsive dose of strychnine abolished postsynaptic inhibition in some neurones, as was evaluated mainly by an increase in repetitive neurone firing (criterion [4]) during stretch of its antagonist muscle. This was the strychnine-sensitive postsynaptic inhibition described by Eccles and co-workers. A majority of the motoneurons in this study, however, showed exactly the opposite response to strychnine that is, an inhibition of the repetitive neu-

rone firing to stretch of its antagonist muscle. This then was another response to strychnine, the strychnine-resistant postsynaptic inhibition. Therefore, a repetitively firing gastrocnemius motoneurone normally shows an inhibition of firing on stretch of the anterior tibialis muscle. In a strychninized cat, this same motoneurone sometimes fired through the period of anterior tibialis stretch (strychnine-sensitive postsynaptic inhibitions), but more often, there were postsynaptic inhibitions which were not sensitive to convulsive doses of strychnine (strychnine-resistant postsynaptic inhibitions).

The experimental results with picrotoxin also showed that the inhibitory mechanisms in the spinal cord cannot be readily categorized. The current view is that picrotoxin blocks presynaptic inhibition, but has no effect on postsynaptic inhibition. Using an experimental approach identical to that used with strychnine, records were obtained from motoneurons in cats injected with convulsive doses of picrotoxin in which repetitive firing persisted during the stretch of their antagonist muscles (picrotoxin-sensitive postsynaptic inhibitions), and from motoneurons in which repetitive firing was inhibited on stretch of the antagonist muscle (the described picrotoxin-resistant postsynaptic inhibitions).

A further indication of the complexity of inhibitory influences on a motoneurone was the response to picrotoxin during muscle stretch in a strychninized cat. After a convulsive dose of strychnine, a majority of neurones showed a strychnine-resistant postsynaptic inhibition. If then a convulsive dose of picrotoxin was injected, this inhibition was converted into an activation during the stretch period, demonstrating a reversal at the postsynaptic membrane not only to strychnine but also to picrotoxin.

Finally, if a motoneurone was impaled with a microelectrode filled with potassium chloride rather than

potassium citrate, chloride ions could be injected into the neurone by applying a hyperpolarizing current through the microelectrode (Coombs, Eccles, and Fatt, 1955; Eccles, Eccles, and Ito, 1964). The effect of this electrophoretic technique is to alter the intracellular chloride concentration, and thereby abolish or reduce the inhibitory postsynaptic potential (IPSP) in strychnine-resistant and picrotoxin-resistant postsynaptic inhibitions. This result is a further indication of the postsynaptic nature of these inhibitions.

CONCLUSIONS

No attempts were made during this study to determine the actual site of action of strychnine or picrotoxin, or the occurrence and distribution of axo-axonal synapses. However, based on the evaluation criteria used, the results suggest that the inhibitory influences on a naturally stimulated spinal cord motoneurone are quite complex and are predominantly postsynaptic in nature. At this time, the influence of presynaptic inhibition on naturally stimulated spinal cord motoneurons is unclear, since EPSP and membrane potential changes have proved to be unreliable as evaluating criteria. Based on more reliable criteria introduced by Granit and co-workers, and in contrast to current views holding that strychnine and picrotoxin could differentiate between presynaptic and postsynaptic inhibition, the present results indicate that no such strict pharmacological differentiation of these inhibitions is possible.

REFERENCES

- COOMBS, J. S., J. C. ECCLES, AND P. FATT. The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory postsynaptic potential. *J. Physiol.* 130: 326–373, 1955.
- ECCLES, J. C. *The Physiology of Synapses*. Berlin, Gottingen, Heidelberg: Springer Verlag, 1964.
- ECCLES, J. C., R. M. ECCLES, AND M. ITO. Effects produced on inhibitory postsynaptic potentials by the coupled injections of cations and anions into motoneurons. *Proc. Roy. Soc. London Ser. B* 160: 197–210, 1964.
- ECCLES, J. C., R. F. SCHMIDT, AND W. D. WILLIS. Pharmacological studies on presynaptic inhibition. *J. Physiol.* 168: 500–530, 1963.
- GRANIT, R., J.-O. KELLERTH, AND T. D. WILLIAMS. Intracellular aspects of stimulating motoneurons by muscle stretch. *J. Physiol.* 174: 435–452, 1964a.
- GRANIT, R., J.-O. KELLERTH, AND T. D. WILLIAMS. 'Adjacent' and 'remote' postsynaptic inhibition in motoneurons stimulated by muscle stretch. *J. Physiol.* 174: 453–472, 1964b.
- KELLERTH, J.-O., AND A. J. SZUMSKI. Two types of stretch-activated postsynaptic inhibitions in spinal motoneurons as differentiated by strychnine. *Acta Physiol. Scand.* 66: 133–145, 1966a.
- KELLERTH, J.-O., AND A. J. SZUMSKI. Effects of picrotoxin on stretch activated postsynaptic inhibitions in spinal motoneurons. *Acta Physiol. Scand.* 66: 146–156, 1966b.